

Emerging and Reemerging Virus Diseases of Blueberry and Cranberry

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Abstract

It should be expected that as blueberry cultivation continues to expand into new areas, the plants will become exposed to viruses that have not previously been observed in blueberry. Since the last symposium in 2004, Blueberry scorch virus continues to be a major concern in the USA Pacific Northwest and it has also been detected in New England as well as the Netherlands and Italy in Europe. In the past, blueberry mosaic symptoms appeared to spread very slowly or not at all, while in recent years, the disease has been spreading within fields and appearing in new fields. Blueberry mosaic symptoms have been observed in many production areas. A high molecular weight double-stranded RNA (dsRNA) has been detected in plants with mosaic symptoms indicating the presence of a virus, but the virus has yet to be characterized. Blueberry red ringspot virus has been detected in the southeastern USA. A virus has been detected in plants exhibiting Blueberry fruit drop symptoms in the Pacific Northwest. In cranberry, funky flower symptoms have been observed in the northeastern USA for the past 10 years. Recently, Cucumber mosaic virus has been detected in plants exhibiting funky flower symptoms. Efforts to demonstrate a causal relationship between Cucumber mosaic virus and disease symptoms are underway. Tobacco streak virus was first reported from cranberry plants imported into Scotland and has since been detected in New Jersey. A breakthrough in detection of viruses in *Vaccinium* species has come from the development of a dsRNA extraction method that works reliably with blueberry and cranberry. Cloning and sequencing of purified dsRNA has been the most successful means for the characterization of viruses of woody plants that can not be transmitted to herbaceous hosts.

INTRODUCTION

There were ten virus and virus-like diseases of blueberry and two of cranberry described in the Compendium of Blueberry and Cranberry Diseases (Caruso and Ramsdell, 1995). Since then, a viroid (Zhu et al., 1995) and two large molecular weight dsRNAs (Martin and Tzanetakis, unpublished data) have been associated with blueberry mosaic disease, a virus in the family *Totiviridae* has been associated with blueberry fruit drop disease (Martin et al., 2006, two viruses in the family *Bromoviridae* have been detected in cranberry (Jones et al., 2001; Caruso et al., 1999), several new virus-like diseases have been observed in blueberry fields and blueberry mosaic-like symptoms have been observed in many growing areas and the disease appears to be spreading rapidly. The most direct way to characterize new viruses that are not transmissible to herbaceous hosts is through the characterization of dsRNAs extracted from diseased plants. DsRNA forms during the replication of RNA viruses and is rarely found in healthy plants, thus its presence is indicative of virus infection. For reasons unknown, standard protocols developed for dsRNA purification (Morris and Dodds, 1979; Valverde et al., 1990) that have been used widely on many different hosts, have not worked with

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blueberry or cranberry tissue. This problem has been overcome with the development of a new dsRNA extraction method that works well with a wide range of hosts (Tzanetakis and Martin, 2008). This method has been used to successfully extract dsRNA from blueberries infected with Blueberry scorch virus (BIScV), mosaic and fruit drop diseases and from cranberry with funky flower symptoms. This provides a means to address new virus-like diseases in *Vaccinium* spp. Due to the low pH of blueberry leaf sap it is critical to control pH of leaf extracts if using ELISA for virus detection in blueberry (MacDonald et al., 1988).

DISCUSSION

Blueberry fruit drop disease (BFD)

Blueberry fruit drop disease was first observed in British Columbia, Canada in the late 1990's and similar symptoms have since been observed in Washington, Oregon and New York. The fruit drop symptom has been dramatic in 'Bluecrop' where nearly 100% of the fruit drops from the bush when the berries are 3-5 mm in diameter. Symptoms of poor fruit set in other cultivars may or may not be related to BFD. 'Bluecrop' plants affected with BFD show a reddening of the young leaves early in the season and a candy stripping of the corolla similar to what is seen with Blueberry shoestring virus. After flowering, the leaves turn green and the bushes appear normal. The fruit drops from the bush about three weeks prior to ripening, when it is about 3-5 mm in diameter. Prior to harvest, affected bushes can be seen from the edge of the field since the fruitless branches are upright and appear taller than neighboring bushes that are laden with fruit. Affected bushes grow more vigorously than fruiting bushes, presumably because they are allocating fewer resources to fruit production and more to vegetative growth. It has not been possible to mechanically transmit the causal organism of BFD to herbaceous hosts using leaf or flower sap, or to graft transmit from blueberry to herbaceous hosts. The disease can, however, be graft transmitted from blueberry to blueberry. In some cases, the disease has spread rapidly within affected fields, in a radial pattern. In other fields the disease appears to spread quite slowly with only a few newly affected bushes per year. The disease has spread to adjacent fields, but a vector has not yet been identified.

A viral dsRNA purified from diseased plants has been cloned and sequenced. The virus is a member of the *Totiviridae* family and most closely related to a virus identified from diseased tomatoes in California, Mississippi and Mexico that has been associated with a stunting and fruit deformation disease. An RT-PCR detection assay has been developed for the BFD associated virus. Using this assay it was shown that the virus is present in fields of symptomless 'Duke' adjacent to infected 'Bluecrop' plantings and also in some symptomless 'Bluecrop' plants. The virus has been detected in Ore., Wash. and British Columbia, but material from New York has not yet been tested. It is unknown if this virus is involved in the BFD, but if it is, it is likely part of a virus complex rather than causing the disease in single infections. With recent improvements in extraction of dsRNA from *Vaccinium* spp. (Tzanetakis and Martin, 2008) efforts to identify other viruses associated with BFD will continue.

Blueberry mosaic disease

Historically, blueberry mosaic has been observed in highbush blueberry plantings in Michigan, Indiana, New Jersey, New York, Oregon, Washington and British Columbia with very little within-field spread observed. Since 2000, this disease has been observed in many blueberry growing areas around the world including, New Zealand, Europe, South Africa, Argentina and Chile (personal observation by RRM from images sent for comment from the countries listed) and appears to be much more common in northern areas of North America, where previously it spread very little. Mosaic affects highbush cultivars 'Bluecrop', 'Pioneer', 'Rubel', 'Cabot', 'Concord', 'Earliblue', 'Jersey', 'Stanley', 'Toro' and occasionally *Vaccinium pallidum* Aiton (a lowbush dryland blueberry). It has not been observed on rabbiteye blueberry. A second 'mosaic-like'

disorder found on almost all plants of 'Coville' may be of genetic origin, though it has not been re-examined with newer methods of virus detection. Fruit on diseased bushes ripen late and are of poor quality. A yield reduction of 15% due to mosaic was reported for 'Bluecrop'. Symptoms on foliage include mild to brilliant mottle and mosaic patterns of yellows, yellow-green, reds or orange. Symptoms may develop erratically on parts of a bush rather than affecting the entire plant. Mosaic symptoms are not produced every year and presumably depend on specific environmental factors, possibly sunlight intensity, day length or temperature, since the disease is usually not seen in southern parts of the USA.

The causal agent of blueberry mosaic is not transmissible by mechanical means or by dodder to herbaceous plants; however, it is graft transmissible. There is a report of a viroid associated with blueberry mosaic (Zhu et al., 1995). Also, dsRNA greater than four kilobases has been detected in blueberry exhibiting mosaic symptoms but the dsRNA has not been further characterized. The size of the dsRNA would suggest a virus as the causal agent. In the case of 'Sunrise', which often develops canes with mosaic symptoms, a dsRNA of different size from typical mosaic diseased plants has been detected (Martin and Tzanetakis, unpublished data). The role of these large dsRNAs or the reported viroid to the mosaic disease needs to be clarified.

Blueberry red ringspot virus (BRRSV)

Red ringspot disease of blueberry was first described from New Jersey in 1950 on highbush blueberry (*V. corymbosum* Dunal). The disease was later determined to be caused by a virus and named Blueberry red ringspot virus (BRRSV). The disease continues to be a problem in New Jersey and has become more widespread in recent years. The disease has now been reported from Arkansas, Michigan, Connecticut, Massachusetts, New York, North Carolina, and Oregon (Caruso and Ramsdell, 1995; Cline et al., 2008). The vector for this disease is unknown and it is interesting to note that the disease does not appear to spread in Michigan. BRRSV is a member of the *Soymovirus* genus of the family *Caulimoviridae*. Antibodies have been developed for detection; however, the virus can also be reliably detected by PCR using BRRSV-specific primers.

Symptoms appear as red rings, 4–6 mm in diameter on one-year old or older stems. Reddish circular spots 3–5 mm in diameter also appear on older leaves in mid- to late summer. These spots are most prominent on the upper surface of the leaf, but in some cultivars, can be visible on the underside of the leaf as well. Circular, light-colored areas of blotching bordered by purple rings, about 2–3 mm in diameter, may develop on affected fruit of some cultivars. Similar symptoms have been seen on stems and fruit of rabbiteye blueberry (*V. ashei* syn. *V. virgatum* Aiton). Some blueberry cultivars, such as 'Ozarkblue', may produce misshapen fruit when infected with BRRSV. 'Bluetta' sometimes exhibits a disorder on leaves only that resembles red ringspot, but this disorder is probably genetic in nature.

A disease similar to BRRSV occurs rarely in American cranberry (*V. macrocarpon* Aiton). Leaf symptoms in cranberry appear as small red blotches. No stem symptoms have been reported. Fruit on affected cranberry plants may exhibit light rings that are best visible as the fruit ripens.

Blueberry scorch virus (BIScV)

A blight disease of unknown etiology of highbush blueberry was observed in New Jersey in the 1970's (Stretch, 1983). BIScV was first described from Washington in 1988 (Martin and Bristow, 1988) and later it was shown that these two diseases were caused by strains of the same virus (Cavaleer et al., 1994). BIScV belongs to the genus *Carlavirus* and is aphid-transmitted in a non-persistent manner. The disease has since been reported in British Columbia, Canada (Bernardy et al., 2004; Wegener et al., 2006), to be more widespread in the northeastern USA (DeMarsay et al., 2004) and in Europe (Ciuffo et al., 2005). The virus is also known to infect other *Vaccinium* species including cranberry (*V. macrocarpon*; Wegener et al., 2004) and wild black huckleberry (*V. membranaceum*

Douglas ex Torrey; Wegener et al., 2007) without any symptoms. Symptomless infections emphasize the importance of including all *Vaccinium* spp. as potential hosts for consideration in quarantine and management decisions. Multiple strains of the virus that differ in symptom expression in some blueberry cultivars have been reported (Cavileer et al., 1994; Bernardy et al., 2004), but in terms of certification or management strategies the differences between strains may be of minor importance as long as the diagnostic method used will detect all strains.

The blueberry aphid, *Ericaphous fimbriata* (Richards), the dominant aphid colonizing highbush blueberry in the Pacific Northwest (PNW; Oregon, Washington and British Columbia) (Raworth, 2004) appears to be the most important vector in this region (Bristow et al., 2000; Raworth, 2004). The aphid population dynamics have been studied and the maximum in field spread of BIScV coincides with the maximum populations of *E. fimbriata* (Bristow et al., 2000; Raworth, 2004). Bristow et al. (2000) suggested that *E. fimbriata* is an inefficient vector of BIScV, which was confirmed by Raworth et al. (2008) where only 2 and 6 out of 119 and 123 trap plants, respectively, with this aphid present, in two separate fields with BIScV, became infected. Important vectors in other areas have not been determined. The virus can be transmitted by a number of other aphids experimentally and the significance of this transmission in the field is unknown. However, it is known that many aphid species migrate across blueberry fields in the spring, even though they do not tend to colonize blueberry. Since the spread of BIScV by aphids is inefficient, it should be possible to manage the virus if one is vigilant in roguing diseased bushes and controlling aphids. Identification of diseased bushes should be based on laboratory tests rather than symptoms since symptom expression varies by blueberry cultivar and virus strain. In addition, the testing needs to be continued for 3-4 years since the virus can be unevenly distributed in a bush and the virus titers can build very slowly over several years (Raworth et al., 2008).

Tobacco streak virus (TSV)

Cranberry vines infected with TSV are symptomless and infected vines were first detected during a routine quarantine screening of imported vines from New Jersey to Scotland for a cultivar screening plot (Jones et al., 2001). 'Pilgrim' and 'AJ' were found to be infected and the original source plantings in NJ continue to have the virus present. Three different strains of TSV were detected, based on the inoculation of herbaceous test plants. To date, there do not appear to be any deleterious effects of the virus on cranberry plants or their production. Tobacco streak virus is the type member of the *Ilarvirus* genus of the *Bromoviridae* family.

Funky flower in Cranberry

In 1997, six cranberry beds in Massachusetts had small areas where flowers were abnormal and did not produce fruits. This condition, termed 'funky flower', has now been found in 20 beds on 18 farms in Massachusetts and seven beds on two farms in New Jersey. It was probably present as far back as the early 1990's in Massachusetts and New Jersey, but growers did not call attention to its occurrence. It has only been observed in 'Early Black', except for one 'Howes' bed in Massachusetts. Affected areas have ranged in size from 0.1 m² (Massachusetts) to 0.12 hectares (New Jersey). In most beds, the affected areas have slowly expanded during the time the beds have been monitored. In some cases, affected areas have coalesced, while in other beds affected vines are intermingled with normal vines.

Two different types of symptoms have been observed (Caruso et al., 1999). In Type A, the petals are contorted and not reflexed with erratic shades of pink (usually darker pink), the stamens are contorted but present, the filaments are reduced in length and the anthers are compressed and misshapen in some instances. The stigma and style are present and appear to be normal, and fruit will occasionally form from selected flowers. In Type B, petals are lacking and the stamens are missing or are severely reduced. The stigma and style are normal but may be shorter than normal, the style is

occasionally turned upward, the pedicel does not curve downward and the flowers are consequently more perpendicular to the stem. Very few fruit form on these flowers, as compared to the Type A flowers. DNA fingerprinting suggested that funky flower is not caused by a genetic mutation affecting a clone that is spreading in established beds. It was thought that there was a genetic mutation that spread by sexual reproduction, but this is unlikely because most affected uprights do not bear fruit.

The condition is carried through propagated cuttings, as well as through the seed produced from the limited number of fruits. The causal agent was shown not to be a phytoplasma (as is false blossom disease in cranberry). Early dsRNA analyses revealed several bands and suggested that the causal entity might be a member of the family *Bromoviridae*. More recently, dsRNA was successfully cloned and regions were sequenced. The sequence data suggests the causal agent is Cucumber mosaic virus, the type member of the genus *Cucumovirus* in the family *Bromoviridae* (Caruso, Tzanetakis, Polashock and Martin, unpublished data). However, it is unclear if this virus alone causes funky flower and is responsible for Type A and Type B symptoms.

SUMMARY

With increased plantings of blueberries and cranberries one would expect to see new virus diseases in these crops for two reasons. There are many examples where crop acreage increases, especially when this has resulted in large areas of monoculture, have led to virus epidemics in annual crops such as rice, maize, soybean or wheat or in perennial crops, such as raspberry, strawberry, citrus or grapevines. In addition, *Vaccinium* crops, especially blueberries, are being planted in many new areas where they are being exposed to wild *Vaccinium* spp., new vectors and viruses. Also, there is an ever increasing pressure on agricultural land and as a result, blueberries and cranberries are being planted adjacent to other horticultural or agronomic crops that may serve as a source of viruses new to *Vaccinium* spp. These more intensive agricultural practices may be the source of new diseases such as funky flower of cranberry or fruit drop of blueberry. For these reasons, it is imperative that virus-like symptoms of these crops be addressed in a timely manner, so that new diseases or developing epidemics can be identified early and managed effectively.

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